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(54) **NUCLEIC ACIDS FOR DETECTING ASPERGILLUS SPECIES AND OTHER FILAMENTOUS FUNGI**

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(58) **Field of Search** 435/6, 91.1; 536/23.1, 536/24.32, 24.3, 23.7

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(57) **ABSTRACT**

Nucleic acids for detecting Aspergillus species and other filamentous fungi are provided. Unique internal transcribed space 2 coding regions permit the development of nucleic acid probes specific for five different species of Aspergillus, three species of Fusarium, four species of Mucor, two species of Penecillium, five species of Rhizopus, one species of Rhizomucor, as well as probes for *Absidia corymbifer, Cunninghamella elagans, Pseudallescheria boydii*, and *Sporothrix schenkii*. Methods are disclosed for the species-specific detection and diagnosis of infection by Aspergillus, Fusarium, Mucor, Penecillium, Rhizomucor, *absidia*, Cunninghaemella, Pseudallescheria or Sporthrix in a subject. Furthermore, genus-specific probes are also provided for Aspergillus, Fusarium and Mucor, in addition to an all-fungus nucleic acid probe.

29 Claims, No Drawings

NUCLEIC ACIDS FOR DETECTING ASPERGILLUS SPECIES AND OTHER FILAMENTOUS FUNGI

PRIORITY CLAIM

This application claims priority to PCT/US98/08926, filed May 1, 1998, which claims the benefit of U.S. Provisional Application No. 60/045,400, filed May 2, 1997.

This invention was made in the Centers for Disease Control Mycotic Diseases Laboratories, an agency of the United States Government.

TECHNICAL FIELD

This application relates in general to the field of diagnostic microbiology. In particular, the invention relates to the species-specific detection of Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Rhizomucor, Absidia, Cunninghamella, *Pseudallescheria boydii* (*Scedosporium apiospermum*), and Sporothrix species.

BACKGROUND OF THE INVENTION

In recent years, chemotherapy for hematological malignancies, and high-dose corticosteroid treatment for organ transplant recipients, along with the spread of AIDS, have greatly increased the number of immunocompromised patients (1, 12, 14, 43). Saprophytic filamentous fungi, such as Aspergillus, Rhizopus, and Mucor species, found in the environment and considered to be of low virulence, are now responsible for an increasing number of infections in the immunocompromised host (17, 20, 43). In addition, these infections are often fulminant and rapidly fatal in immunocompromised patients (7, 11, 12, 20, 44). Morbidity and mortality is extremely high; for example, aspergillosis has a mortality rate of approximately 90% (8, 11).

To complicate matters, diagnosis is difficult and symptoms are often non-specific (18, 27, 29, 42, 44). Antibody-based tests can be unreliable due to the depressed or variable immune responses of immunocompromised patents (2, 9, 18, 46). Antigen detection tests developed to date have fallen short of the desired sensitivity (2, 9, 38). Radiographic evidence can be non-specific and inconclusive (5, 29, 36), although some progress in diagnosis has been made with the advent of computerized tomography (40). However, definitive diagnosis still requires either a positive blood or tissue culture or histopathological confirmation (3, 21). An added complication is that the invasive procedures necessary to obtain biopsy materials are often not recommended in thrombocytopenic patient populations (37, 41).

Even when cultures of blood, lung or rhinocerebral tissues are positive, morphological and biochemical identification of filamentous fungi can require several days for adequate growth and sporulation to occur, delaying targeted drug therapy. Some atypical isolates may never sporulate, making identification even more difficult (23). When histopathology is performed on tissue biopsy sections, the morphological similarities of the various filamentous fungi in tissue make differentiation difficult (16). Fluorescent antibody staining of histopathological tissue sections is not specific unless cross-reactive epitopes are absorbed out which can make the resultant antibody reactions weak (14, 19). Therapeutic choices vary (7, 41, 44) making a test to rapidly and specifically identify filamentous fungi urgently needed for the implementation of appropriately targeted therapy. Early and accurate diagnosis and treatment can decrease morbidity and increase the chances for patient survival (6, 27, 39).

Furthermore, identification of filamentous fungi to at least the species level would be epidemiologically useful (24, 31, 43, 47).

PCR-based methods of detection, which show promise as rapid, sensitive means to diagnose infections, have been used in the identification of DNA from Candida species (13, 15, 30) and some other fungi, particularly Aspergillus species (31, 33, 45). However, most of these tests are only genus-specific (28, 38) or are directed to detect only single-copy genes (4, 35). Others have designed probes to detect multi-copy genes so as to increase test sensitivity (31, 33) but in doing so have lost test specificity because they have used highly conserved genes, which detect one or a few species but which are also plagued with cross-reactivities to human, fungal or even viral DNA (25, 31, 33).

Therefore, it is an object of the invention to provide improved materials and methods for detecting and differentiating Aspergillus and other filamentous fungal species in the clinical and laboratory settings.

SUMMARY OF THE INVENTION

The present invention relates to nucleic acids for detecting Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Rhizomucor, Absidia, Cunninghamella, Pseudallescheria (Scedosporium), and Sporothrix species. Unique internal transcribed spacer 2 coding regions permit the development of probes specific for five different Aspergillus species, *A. flavus, A. fumigatus, A. niger, A. terreus*, and *A. nidulans*. The invention thereby provides methods for the species-specific detection and diagnosis of Aspergillus infection in a subject. In addition, species probes have been developed for three Fusarium, four Mucor, two Penicillium, five Rhizopus and one Rhizomucor species, as well as probes for *Absidia corymbifera, Cunninghamella elegans, Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenckii*. Generic probes for Aspergillus, Fusarium, and Mucor species have also been developed.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiments and the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a simple, rapid, and useful method for differentiating filamentous fungal species from each other and from other medically important fungi. This invention enables a rapid, simple and useful method to isolate fungal DNA from host samples, and to apply the species- and genus-specific probes for the diagnosis of a disease. Ultimately, these probes can be used for in situ hybridization or in situ PCR diagnostics so that the morphology of host tissue, and microorganisms, remain intact.

The invention provides nucleic acids containing regions of specificity for five Aspergillus, three Fusarium, four Mucor, two Penicillium, five Rhizopus and one Rhizomucor species as well as probes for *Absidia corymbifera, Cunninghamella elegans, Pseudallescheria boydii* (*Scedosporium apiospremum*), and *Sporothrix schenckii*. These nucleic acids are from the internal transcribed spacer 2 ("ITS2") region of ribosomal deoxyribonucleic acid (rDNA) of the genome of the aforementioned filamentous fungi. The ITS2 region is located between the 5.8S rDNA region and the 28S rDNA region.

In particular, the invention provides nucleic acids from *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus*

(SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), *Aspergillus nidulans* (SEQ ID NO:5), *Fusarium solani* (SEQ ID NO:6), *Fusarium moniliforme* (SEQ ID NO:7), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO: 10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsportis* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18). *Rhizopus stolonifer* (SEQ ID NO:19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), and *Sporothrix schenkii* (SEQ ID NO:29). These sequences can be used to identify and distinguish the respective species of Aspergillus, Fusariunm, Mucor, Rhizopus, and Penicillium, and identify and distinguish these species from each other and from *Absidia corymbifera, Cunninghamella elegans, Pseudallescheria boydii*(*Scedosporium apiospermum*), and *Sporothrix schenkii.*

Furthermore, the invention provides isolated nucleic acid probes derived from GenBank nucleic acid sequences (for *Penicillium marneffei* and *Fusarium oxysporum* only) or from the above nucleic acid sequences which may be used as species-specific identifiers of *Aspergillus flavus* (SEQ ID NO:30 and 31), *Aspergillus fumigatus* (SEQ ID NO:32), *Aspergillus niger* (SEQ ID NO:33), *Aspergillus terreus* (SEQ ID NO:34), *Aspergillus nidulans* (SEQ ID NO:35), *Mucor rouxii* (SEQ ID NO:36), *Mucor plumbeus* (SEQ ID NO:37), *Mucor indicus* (SEQ ID NO:38), *Mucor circinilloides f. circinelloides* (SEQ ID NO:39), *Mucor racemosus* (SEQ ID NO:40), *Rhizopus oryzae* (SEQ ID NO:41), *Rhizopus circinans* (SEQ ID NO:42), *Rhizomucor pusillus* (SEQ ID NO:43), *Rhizopus stolonifer* (SEQ ID NO:44), *Pseudallescheria boydii* (*Scedosporium apiospermumn*)(SEQ ID NO:45), *Penicillium notatum* (SEQ ID NO:46), *Penicillium marneffei* (SEQ ID NO:47 and 48), *Fusarium moniliforme* (SEQ ID NO:49), *Fusarium oxysporum* (SEQ ID NO:50), *Fusarium solani* (SEQ ID NO:51), *Cunninghamella elegans* (SEQ ID NO:52, 53, and 54), *Absidia corymbifera* (SEQ ID NO:55), *Sporothrix schenkii* (SEQ ID NO:56), and *Rhizopus microsporus* (SEQ ID NO:57). Such probes can be used to selectively hybridize with samples containing nucleic acids from species of Aspergillus, Fusarium, Mucor, Rhizopus (or Rhizomucor), Penicillium, or from *Absidia corymbifera, Cunninghamella elegans, Pseudallescheria boydii* (*Scedosporium apiospermum*), and *Sporothrix schenkii*. These fungi can be detected after polymerase chain reaction or ligase chain reaction amplification of fungal DNA and specific probing of amplified DNA with DNA probes labeled with digoxigenin, reacted with anti-digoxigenin antibodies labeled with horseradish peroxidase and a colorimetric substrate, for example. Additional probes can routinely be derived from the sequences given in SEQ ID NOs: 1–29, which are specific for the respective species. Therefore, the probes shown in SEQ ID NOs:30–57 are only provided as examples of the species-specific probes that can be derived from SEQ ID NOs: 1–29.

Generic probes for Aspergillus (SEQ ID NO:58), Fusarium, (SEQ ID NO:59) and Mucor (SEQ ID NO:60) species have also been developed to identify all members of their respective species which are listed above as well as an all-fungus biotinylated probe (SEQ ID NO:61) to capture all species-specific and generic probes listed above for their detection.

By "isolated" is meant nucleic acid free from at least some of the components with which it naturally occurs. By "selective" or "selectively" is meant a sequence which does not hybridize with other nucleic acids to prevent adequate determination of an Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus or Rhizomucor genus or species or of *Absidia corymbifera, Cunninghamella elegans, Pseudallescheria boydii* (*Scedosporium apiospermum*), or *Sporothrix schenckii* species.

The hybridizing nucleic acid should have at least 70% complementarity with the segment of the nucleic acid to which it hybridizes. As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids and thus has the same meaning as "specifically hybridizing". The selectively hybridizing nucleic acids of the invention can have at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, and 99% complementarity with the segment of the sequence to which it hybridizes.

The invention contemplates sequences, probes and primers which selectively hybridize to the complementary, or opposite, strand of DNA as those specifically provided herein. Specific hybridization with nucleic acid can occur with minor modifications or substitutions in the nucleic acid, so long as functional species-specific or genus-specific hybridization capability is maintained. By "probe" is meant nucleic acid sequences that can be used as probes or primers for selective hybridization with complementary nucleic acid sequences for their detection or amplification, which probes can vary in length from about 5 to 100 nucleotides, or preferably from about 10 to 50 nucleotides, or most preferably about 18 nucleotides. The invention provides isolated nucleic acids that selectively hybridize with the species-specific nucleic acids under stringent conditions and should have at least 5 nucleotides complementary to the sequence of interest. See generally, Maniatis (26).

If used as primers, the invention provides compositions including at least two nucleic acids which hybridize with different regions so as to amplify a desired region. Depending on the length of the probe or primer, target region can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of the Aspergillus, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (e.g., Aspergillus DNA from a sample) is at least enough to distinguish hybridization with a nucleic acid from other yeasts and filamentous fungi. The invention provides examples of nucleic acids unique to each filamentous fungus in the listed sequences so that the degree of complementarity required to distinguish selectively hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid.

Alternatively, the nucleic acid probes can be designed to have homology with nucleotide sequences present in more than one species of the fungi listed above. Such a nucleic acid probe can be used to selectively identify a group of species such as the generic probes listed for Aspergillus (SEQ ID NO:58), Fusarium (SEQ ID NO:59), and Mucor (SEQ ID NO:60) as well as all fungi listed (SEQ ID NO:61). Additionally, the invention provides that the nucleic acids can be used to differentiate the filamentous fungi listed in general from other filamentous fungi and yeasts, such as Candida species. Such a determination is clinically significant, since therapies for these infections differ.

The invention further provides methods of using the nucleic acids to detect and identify the presence of the

filamentous fungi listed, or particular species thereof. The method involves the steps of obtaining a sample suspected of containing filamentous fungi. The sample may be taken from an individual, such as blood, saliva, lung lavage fluids, vaginal mucosa, tissues, etc., or taken from the environment. The filamentous fungal cells can then be lysed, and the DNA extracted and precipitated. The DNA is preferably amplified using universal primers derived from the internal transcribed spacer regions, 18S, 5.8S and 28S regions of the filamentous fungal rDNA. Examples of such universal primers are shown below as ITS1 (SEQ ID NO:62), ITS3 (SEQ ID NO:63), ITS4 (SEQ ID NO:64). Detection of filamentous fungal DNA is achieved by hybridizing the amplified DNA with a species-specific probe that selectively hybridizes with the DNA. Detection of hybridization is indicative of the presence of the particular genus (for generic probes) or species (for species probes) of filamentous fungus.

Preferably, detection of nucleic acid (e.g. probes or primers) hybridization can be facilitated by the use of detectable moieties. For example, the species-specific or generic probes can be labeled with digoxigenin, and an all-fungus probe, such as described in SEQ ID NO:61, can be labeled with biotin and used in a streptavidin-coated microtiter plate assay. Other detectable moieties include radioactive labeling, enzyme labeling, and fluorescent labeling, for example.

The invention further contemplates a kit containing one or more species-specific probes, which can be used for the detection of particular filamentous fungal species and genera in a sample. Such a kit can also contain the appropriate reagents for hybridizing the probe to the sample and detecting bound probe. The invention may be further demonstrated by the following non-limiting examples.

EXAMPLES

In this example, PCR assay employing universal, fungus-specific primers and a simple, rapid EIA-based format for amplicon detection were used.

Extraction of Filamentous Fungal DNA

A mechanical disruption method was used to obtain DNA from filamentous fungal species and an enzymatic disruption method described previously (13) was used to obtain DNA from yeasts. Filamentous fungi were grown for 4 to 5 days on Sabouraud dextrose agar slants (BBL, division of Becton Dickinson, Cockeysville, Md.) at 35° C. Two slants were then washed by vigorously pipeting 5 mls of 0.01 M potassium phosphate buffered saline (PBS) onto the surface of each slant and the washes were transferred to 500 ml Erlenmeyer flasks containing 250 ml of Sabouraud dextrose broth (BBL). Flasks were then incubated for 4 to 5 days on a rotary shaker (140 rpm) at ambient temperature. Growth was then harvested by vacuum filtration through a sterile Whatman #1 filter paper which had been placed into a sterile Buchner funnel attached to a 2 L side-arm flask. The resultant cellular mat was washed on the filtration apparatus three times with sterile distilled water, removed from the filter paper by gentle scraping with a rubber policeman, and placed into a sterile Petri plate which was then sealed with parafilm and frozen at −20° C. until used.

Just prior to use, a portion of the frozen cellular mat, equal in size to a quarter, was removed and placed into a cold mortar (6" diameter). Liquid nitrogen was added to cover the mat which was then ground into a powder with a pestle. Additional liquid nitrogen was added as needed to keep the mat frozen during grinding.

DNA was then purified using proteinase K and RNase treatment, multiple phenol extractions, and ethanol precipitation by conventional means (26).

PCR amplification

The fungus-specific, universal primer pair ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3') (SEQ ID NO:63) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (SEQ ID NO:64) was used to amplify a portion of the 5.8S rDNA region, the entire ITS2 region, and a portion of the 28S rDNA region for each species as previously described (13, 34). DNA sequencing used this primer pair and also the fungus-specific, universal primer pair ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') (SEQ ID NO: 62) and ITS4 to amplify a portion of the 18S rDNA region, the entire 5.8S region, the entire ITS1 and ITS2 regions, and a portion of the 28S rDNA region.

A DNA reagent kit (TaKaRa Biomedicals, Shiga, Japan) was used for PCR amplification of genomic DNA. PCR was performed using 2 μl of test sample in a total PCR reaction volume of 100 μl consisting of 10 μl of 10×Ex Tag buffer, 2.5 mM each of dATP, dGTP, dCTP, and dTTP, in 8 μl 0.2 μM of each primer, and 0.5 U of TaKaRa Ex Tag DNA polymerase. Thirty cycles of amplification were performed in a Perkin-Elmer 9600 thermal cycler (Emeryville, Calif.) after initial denaturation of DNA at 95° C. for 5 minutes. Each cycle consisted of a denaturation step at 95° C. for 30 seconds, an annealing step at 58° C. for 30 seconds, and an extension step at 72° C. for 1 minute. A final extension at 72° C. for 5 minutes followed the last cycle. After amplification, samples were stored at −20° C. until used.

TABLE 1

Synthetic Universal Oligonucleotides Used in PCR and Hybridization Analyses

Primers or Probes	Nucleotide Sequence (5' to 3')	Chemistry and Location
ITS3	GCA TCG ATG AAG AAC GCA GC (SEQ ID NO:63)	5.85 rDNA universal 5' primer
ITS4	TCC TCC GCT TAT TGA TAT GC (SEQ ID NO:64)	28S rDNA universal 3' primer
ITSI	TCC GTA GGT GAA CCT GCG G (SEQ ID NO:62)	185 rDNA universal 5' primer

DNA Sequencing

Primary DNA amplifications were conducted as described above. The aqueous phase of the primary PCR reaction was purified using QIAquick Spin Columns (Quiagen, Chatsworth, Calif.). DNA was eluted from each column with 50 μl of heat-sterilized Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

Purified DNA was labeled using a dye terminator cycle sequencing kit (ABI PRISM, Perkin Elmer, Foster City, Calif.). One mix was made for each of the primers so that sequencing could be performed in both the forward and reverse directions. The reaction volume (20 μl) contained 9.5 μl Terminator Premix, 2 μl (1 ng) DNA template, 1 μl primer (3.2 pmol) and 7.5 μl heat-sterilized distilled H_2O. The mixture was then placed into a pre-heated (96° C.) Perkin Elmer 9600 thermal cycler for 25 cycles of 96° C. for 10 seconds, 50° C. for 5 seconds, 60° C. for 4 minutes. The PCR product was then purified before sequencing using CentriSep spin columns (Princeton Separations, Adelphia, N.J.). DNA was then vacuum dried, resuspended in 6 μl of formamide-EDTA (5 μl deionized formamide plus 1 μl 50 mM EDTA, pH 8.0), and denatured for 2 min at 90° C. prior to sequencing using an automated capillary DNA sequencer (ABI Systems, Model 373, Bethesda, Md.).

The sequencing results were as follows:

Aspergillus flavus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:1)

GCTGCCCATC AAGCACGGC TTGTGTGTTG GGTCGTCGTC

CCCTCTCCGG GGGGGACGGG CCCCAAAGGC AGCGGCGGCA

CCGCGTCCGA TCCTCGAGCG TATGGGGCTT TGTCACCCGC

TCTGTAGGCC CGGCCGGCGC TTGCCGAACG CAAATCAATC

TTTTTCCAGG TTGACCTCGG ATCAGGTAGG GATACCCGCT

GAACTTCAA

Aspergillus fumigatus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:2)

AAACTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA

AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA

TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC

CTGGTATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT

GCCCATCAAG CACGGCTTGT GTGTTGGGCC CCCGTCCCCC

TCTCCCGGGG GACGGGCCCG AAAGGCAGCG GCGGCACCGC

GTCCGGTCCT CGAGCGTATG GGGCTTGTCA CCTGCTCTGT

AGGCCCGGCC GGCGCCAGCC GACACCCAAC TTTATTTTTC

TAAGGTTGAC CTCGGATCAG GTAGGGATAC CCGCTGAACT TAAA

Aspergillus niger 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:3)

AAACTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA

AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA

TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC

CTGGTATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT

GCCCTCAAGC ACGGCTTGTG TGTTGGGTCG CCGTCCCCCT

CTCCCGGGGG ACGGGCCCGA AAGGCAGCGG CGGCACCGCG

TCCGATCCTC GAGCGTATGG GGCTTTGTCA CCTGCTCTGT

AGGCCCGGCC GGCGCCTGCC GACGTTATCC AACCATTTTT

TTCCAGGTTG ACCTCGGATC AGGTAGGGAT ACCCGCTGAA CTTAA

Aspergillus terreus 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:4)

AAACTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA

AGAACGCAGC GAAATGCGAT AACTAATGTG AATTGCAGAA

TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC

-continued

CTGGTATTCC GGGGGGGCAT GCCTGTCCGA GCGTCATTGC

TGCCCTCAAG CCCGGCTTGT GTGTTGGGCC CTCGTCCCCC

GGCTCCCGGG GGACGGGCCC GAAAGGCAGC GGCGGCACCG

CGTCCGGTCC TCGAGCGTAT GGGGCTTCGT CTTCCGCTCC

GTAGGCCCGG CCGGCGCCCG CCGAACGCAT TTATTTGCAA

CTTGTTTTTT TTTCCAGGTT GACCTCGGAT CAGGT

Aspergillus nidulans 5.8S ribosomal RNA gene, partial sequence, internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence.

(SEQ ID NO:5)

AAACTTTCAA CAATGGATCT CTTGGTTCCG GCATCGATGA

AGAACGCAGC GAACTGCGAT AAGTAATGTG AATTGCAGAA

TTCAGTGAAT CATCGAGTCT TTGAACGCAC ATTGCGCCCC

CTGGCATTCC GGGGGGCATG CCTGTCCGAG CGTCATTGCT

GCCCTCAAGC CCGGCTTGTG TGTTGGGTCG TCGTCCCCCC

CCCCGGGGGA CGGGCCCGAA AGGCAGCGGC GGCACCGGTC

CGGTCCTCGA GCGTATGGGG CTTGGTCACC CGCTCGATTA

GGGCCGGCCG GGCGCCAGCC GGCGTCTCCA ACCTTATCTT

TCTCAGGTTG ACCTCGGATC AGGTAGGGAT ACCCGCTGAA CTTAA

Fusarium solani (strain ATCC62877) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:6)

GAAAATGCGA TAAGTAATGT GAATTGCAGA ATTCAGTGAA

TCATCGAATC TTTGAACGCA CATTGCGCCC GCCAGTATTC

TGGCGGGCAT GCCTGTTCGA GCGTCATTAC AACCCTCAGG

CCCCCGGGCC TGGCGTTGGG GATCGGCGGA AGCCCCCTGC

GGGCACAACG CCGTCCCCCA AATACAGTGG CGGTCCCGCC

GCAGCTTCCA TTGCGTAGTA GCTAACACCT CGCAACTGGA

GAGCGGCGCG GCCACGCCGT AAAACACCCA ACTTCTGAAT

GTTGACCTCG AATCAGGTAG GAATACCCGC TGAACTTAA

Fusarium moniliforme (strain ATCC38519) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:7)

AAATGCGATA AGTAATGTGA ATTGCAAAAT TCAGTGAATC

ATCGAATCTT TGAACGCACA TTGCGCCCGC CAGTATTCTG

GCGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCC

CCCGGGTTTG GTGTTGGGGA TCGGCAAGCC CTTGCGGCAA

GCCGGCCCCG AAATCTAGTG GCGGTCTCGC TGCAGCTTCC

ATTGCGTAGT AGTAAAACCC TCGCAACTGG TACGCGGCGC

-continued

GGCCAAGCCG TTAAACCCCC AACTTCTGAA TGTTGACCTC

GGATCAGGTA GGAATACCCG CTGAACTTAA

Mucor rouxii (strain ATCC24905) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:8)

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA

ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC

CAGCATTTTG TTGAATAGGA ATACTGAGAG TCTCTTGATC

TATTCTGATC TCGAACCTCT TGAAATGTAC AAAGGCCTGA

TCTTGTTTAA ATGCCTGAAC TTTTTTTTAA TATAAAGAGA

AGCTCTTGCG GTAAACTGTG CTGGGGCCTC CCAAATAATA

CTCTTTTTAA ATTTGATCTG AAATCAGGCG GGATTACCCG

CTGAACTTAA

Mucor racemosus (strain ATCC22365) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:9)

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA

ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC

CAACTTTTGT TGTATAGGAT TATTGGGGGC CTCTCGATCT

GTATAGATCT TGAAATCCCT GAAATTTACT AAGGCCTGAA

CTTGTTTAAA TGCCTGAACT TTTTTTTAAT ATAAAGGAAA

GCTCTTGTAA TTGACTTTGA TGGGGCCTCC CAAATAAATC

TCTTTTAAAT TTGATCTGAA ATCAGGCGGG ATTACCCGCT

GAACTTAA

Mucor plumbeus (strain ATCC4740) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:10)

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA

ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC

CAACTTTTGT TGTATAGGAT TATTGGGGGC CTCTCGATCT

GTATAGATCT TGAAACCCTT GAAATTTACT AAGGCCTGAA

CTTGTTTAAT GCCTGAACTT TTTTTTAATA TAAAGGAAAG

CTCTTGTAAT TGACTTTGAT GGGGCCTCCC AAATAAATCT

TTTTTAAATT TGATCTGAAA TCAGGTGGGA TTACCCGCTG

AACTTAA

Mucor indicus (strain ATCC4857) internal transcribed spacer 2 and adjacent regions.

(SEQ ID NO:11)

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCATC TTGCACTCAA TGGTATTCCA

TTGAGTACGC CTGTTTCAGT ATCAAAAAC AACCCTTATT

CAAAATTCTT TTTTTGAATA GATATGAGTG TAGCAACCTT

ACAAGTTGAG ACATTTTAAA TAAAGTCAGG CCATATCGTG

GATTGAGTGC CGATACTTTT TTAATTTTGA AAAGGTAAAG

CATGTTGATG TCCGCTTTTT GGGCCTCCCA AATAACTTTT

TAAACTTGAT CTGAAATCAG GTGGGATTAC CCGCTGAACT

TAA

Mucor circinelloides f. circinelloides (strain ATCC1209B) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCAAC TTGCGCTCAT TGGTATTCCA

ATGAGCACGC CTGTTTCAGT ATCAAAACAA ACCCTCTATC

CAACATTTTT GTTGAATAGG ATGACTGAGA GTCTCTTGAT

CTATTCTGAT CTCGAAGCTC TTGAAATGTA CAAAGGCCTG

ATCTTGTTTG AATGCCTGAA CTTTTTTTTA ATATAAAGAG

AAGCTCTTGC GGTAAACTGT GCTGGGGCCT CCCAAATAAC

ACATCTTTAA ATTTGATCTG AAATCAGGT GGGACTACCC

GCTGAACTT AA (SEQ ID NO:12)

Rhizopus oryzae (strain ATCC34965) internal transcribed spacer 2 and adjacent regions.

AGTGCGATAA CTAGTGTGAA TTGCATATTC AGTGAATCAT

CGAGTCTTTG AACGCAGCTT GCACTCTATG GTTTTTCTAT

AGAGTACGCC TGCTTCAGTA TCATCACAAA CCCACACATA

ACATTTGTTT ATGTGGTGAT GGGTCGCATC GCTGTTTTAT

TACAGTGAGC ACCTAAAATG TGTGTGATTT TCTGTCTGGC

TTGCTAGGCA GGAATATTAC GCTGGTCTCA GGATCTTTTT

TTTTGGTTCG CCCAGGAAGT AAAGTACAAG AGTATAATCC

AGTAACTTTC AAACTATGAT CTGAAGTCAG GTGGGATTAC

CCGCTGAACT TAA (SEQ ID NO:13)

Rhizopus oryzae (strain ATCC11886) internal transcribed spacer 2 and adjacent regions.

AGTGCGATAA CTAGTGTGAA TTGCATATTC AGTGAATCAT

CGAGTCTTTG AACGCAGCTT GCACTCTATG GTTTTTCTAT

AGAGTACGCC TGCTTCAGTA TCATCACAAA CCCACACATA

ACATTTGTTT ATGTGGTAAT GGGTCGCATC GCTGTTTTAT

TACAGTGAGC ACCTAAAATG TGTGTGATTT TCTGTCTGGC

-continued

TTGCTAGGCA GGAATATTAC GCTGGTCTCA GGATCTTTTT

CTTTGGTTCG CCCAGGAAGT AAAGTACAAG AGTATAATCC

AGCAACTTTC AAACTATGAT CTGAAGTCAG GTGGGATTAC

CCGCTGAACT TAA (SEQ ID NO:14)

Rhizopus microsporus (strain ATCC14056) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCGTGAATCA

TCGAGTCTTT GAACGCAGCT TGCACTCTAT GGATCTTCTA

TAGAGTACGC TTGCTTCAGT ATCATAACCA ACCCACACAT

AAAATTTATT TTATGTGGTG ATGGACAAGC TCGGTTAAAT

TTAATTATTA TACCGATTGT CTAAAATACA GCCTCTTTGT

AATTTTCATT AAATTACGAA CTACCTAGCC ATCGTGCTTT

TTTGGTCCAA CCAAAAAACA TATAATCTAG GGGTTCTGCT

AGCCAGCAGA TATTTTAATG ATCTTTAACT ATGATCTGAA

GTCAAGTGGG ACTACCCGCT GAACTTAA (SEQ ID NO:15)

Rhizopus microsporus (strain ATCC12276) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCGTGAATCA

TCGAGTCTTT GAACGCAGCT TGCACTCTAT GGATCTTCTA

TAGAGTACGC TTGCTTCAGT ATCATAACCA ACCCACACAT

AAAATTTATT TTATGTGGTG ATGGACAAGC TCGGTTAAAT

TTAATTATTA TACCGATTGT CTAAAATACA GCCTCTTTGT

AATTTTCATT AAATTACGAA CTACCTAGCC ATCGTGCTTT

TTTGGTCCAA CCAAAAAACA TATAATCTAG GGGTTCTGCT

AGCCAGCAAA TATTTTAATG ATCTTTAACC TATGATCTGA

AGTCAAGTGG GACTACCCGC TGAACTTAA (SEQ ID NO:16)

Rhizopus circinans (strain ATCC34106) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAT AACTAGTGTG AATTGCATTT TCAGTGAATC

ATCGAGTCTT TGAACGCAT CTTGCGCTCT TGGGATTCTT

CCCTAGAGCA CACTTGCTTC AGTATCATAA CAAAACCCTC

ACCTAATATT TTTTTTTTTT AAAAAAAAAA TATTAGAGTG

GTATTGGGGT CTCTTTGGTA ATTCTTTGTA ATTATAAAAG

TACCCTTAAA TGTCATAAAC AGGTTAGCTT TAGCTTGCCT

TTAAAGATCT TCTTAGGGTA TCATTACTTT TCGTAAATCT

TTAATAGGCC TGTCACATAA TTCTACCCTT AAATTTCTTA

AACCTTGATC TGAAGTCAAG TGGGAGTACC CGCTGAACTT AA

(SEQ ID NO:17)

Rhizopus circinans (strain ATCC34101) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAT AACTAGTGTG AATTGCATTT TCAGTGAATC

ATCGAGTCTT TGAACGCATC TTGCGCTCTT GGGATTCTTC

CCTAGAGCAC ACTTGCTTCA GTATCATAAC AAAACCCTCA

CCTAATATTT TTTTTTAAAA AAAAAAAATA TTAGAGTGGT

ATTGGGGTCT CTTTGGTAAT TCTTTGTAAT TATAAAAGTA

CCCTTAAATG TCATAAACAG GTTAGCTTTA GCTTGCCTTT

AAAGATCTTC TTAGGGTATC ATTACTTTTC GTAAATCTTT

AATAGGCCTG TCACATAATT CTACCCTTAA ATTTCTTAAA

CCTTGATCTG AAGTCAAGTG GGAGTACCCG CTGAACTTAA

(SEQ ID NO:18)

Rhizous stolonifer (strains ATCC14037 and 6227A) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AACTAGTGTG AATTGCATAT TCAGTGAATC

ATCGAGTCTT TGAACGCAAC TTGCACTCTA TGGTTTTCCG

TAAAGTACGC TTGCTTCAGT ATCATAAAGA CCCCATCCTG

ATTATTATTT TTTTATTAAA ATAATTAATT TTGGAGATAA

TAAAAATGAG GCTCTTTCTT TTCTTTTTTT TTTTTTTAAA

AAAAAGGGGG GGAAAGGGTC TTTTAAAATG GGCAAATTCT

GGGTTTTTTA CTAAACCTGA ACTCCCCCCA AAAATTCAAA

AAAAAAAAAA TGGGTTTTAC CAAATTTTTT TTTTTTTTCT

CCTTTTTGTG TAGTTAATAC TCTATTAAAT TTATTTACTT

GGTATTATAA CGATTATGCA AGAAGGGAGA GAACAAAGAA

TAATGAAAGA GAGTTTTTAA ATAAATTCTT TTTTCATTTT

TTCAATCAAT GATCTGAAGT CAAGTGGGAT TACCCGCTGA

ACTTAA (SEQ ID NO:19)

Rhizomucor pusillus (strain ATCC36606) internal transcribed spacer 2 and adjacent regions.

AAATTGCGAA AAGTAATGCG ATCTGCAGCC TTTGCGAATC

ATCGAATTCT CGAACGCACC TTGCACCCTT TGGTTCATCC

ATTGGGTACG TCTAGTTCAG TATCTTTATT AACCCCTAAA

GGTTTATTTT TTGATAAATC TTTGGATTTG CGGTGCTGAT

GGATTTTCAT CCGTTCAAGC TACCCGAACA ATTTGTATGT

TGTTGACCCT TGATATTTCC TTGAGGGCTT GCATTGGTAT

CTAATTTTTT ACCAGTGTGC TTCGAGATGA TCAAGTATAA

AGGTCAATCA ACCACAAATA AATTTCAACT ATGGATCTGA

ACTTAGATGG GATTACCCGC TGAACTTAA (SEQ ID NO:20)

Absidia corymbifera (strain ATCC46774) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AATTATTGCG ACTTGCATTC ATAGCGAATC
ATCGAGTTCT CGAACGCATC TTGCGCCTAG TAGTCAATCT
ACTAGGCACA GTTGTTTCAG TATCTGCAAC TACCAATCAG
TTCAACTTGG TTCTTTGAAC CTAAGCGAGC TGGAAATGGG
CTTGTGTTGA TGGCATTCAG TTGCTGTCAT GGCCTTAAAT
ACATTTAGTC CTAGGCAATT GGCTTTAGTC ATTTGCCGGA
TGTAGACTCT AGAGTGCCTG AGGAGCAACG ACTTGGTTAG
TGAGTTCATA ATTCCAAGTC AATCAGTCTC TTCTTGAACT
AGGTCTTAAT CTTTATGGAC TAGTGAGAGG ATCTAACTTG
GGTCTTCTCT TAAAACAAAC TCACATCTAG ATCTGAAATC
AACTGAGATC ACCCGCTGAA CTTAA (SEQ ID NO:21)

Absidia corymbifera (strain ATCC46773) internal transcribed spacer 2 and adjacent regions.

AAAGTGCGAT AATTATTGCG ACTTGCATTC ATAGTGAATC
ATCGAGTTCT TGAACGCATC TTGCGCCTAG TAGTCAATCT
ACTAGGCACA GTTGTTTCAG TATCTGCATC CACCAATCAA
CTTAACCTTT TGTGTTGAGT TGGAACTGGG CTTCTAGTTG
ATGGCATTTA GTTGCTGTCA TGGCCTTAAA TCAATGTCCT
AGGTGTTAGA ACATCTAACA CCGGATGGAA ACTTTAGAGC
GCTTTAAGAG CAGCTTGGTT AGTGAGTTCA ATAATTCCAA
GCATTAAGTC TTTTAATGAA CTAGCTTTTC TATCTATGGG
ACACTACTTG GAGAAATCCA AGTAACCTTT AAACTCCCAT
TTAGATCTGA AATCAACTGA GACCACCCGC TGAACTTAA
(SEQ ID NO:22)

Cunninghamella elegans (strain ATCC42113) internal transcribed spacer 2 and adjacent regions.

AAATCGCGAT ATGTAATGTG ACTGCCTATA GTGAATCATC
AAATCTTTGA AACGCATCTT GCACCTTATG GTATTCCATA
AGGTACGTCT GTTTCAGTAC CACTAATAAA TCTCTCTCTA
TCCTTGATGA TAGAAAAAAA AAAAATAATT TTTACTGGGC
CCGGGGAATC CTTTTTTTTT TTTAATAAAA AGGACCAATT
TTGGCCCAAA AAAAAGGGTT GAACTTTTTT TACCAGATCT
TGCATCTAGT AAAAACCTAG TCGGCTTTAA TAGATTTTTA
TTTTCTATTA AGTTTATAGC CATTCTTATA TTTTTTAAAA
TCTTGGCCTG AAATCAGATG GGATACCCGC TGAACTTAA
(SEQ ID NO:23)

Pseudallescheria boydii (strain ATCC44328) internal transcribed spacer 2 and adjacent regions (teleomorph of *Scedosporium apiospennum*).

AAATGCGATA AGTAATGTAA ATTGCAAAAT TCAGTGAATC
ATCGAATCTT TGAAACGCAC ATTGCGCCCG GCAGTAATCT
GCCGGGCATG CCTGTCCGAG CGTCATTTCA ACCCTCGAAC
CTCCGTTTC CTTAGGGAAG CCTAGGGTCG GTGTTGGGGC
GCTACGGCAA GTCCTCGCAA CCCCCGTAGG CCCTGAAATA
CAGTGGCGGT CCCGCCGCGG TTGCCTTCTG CGTAGTAAGT
CTCTTTTGCA AGCTCGCATT GGGTCCCGGC GGAGGCCTGC
CGTCAAACCA CCTAACAACT CCAGATGGTT TGACCTCGGA
TCAGGTAGGG TTACCCGCTG AACTTAA (SEQ ID NO:24)

Pseudallescheria boydii (strain ATCC36282) internal transcribed spacer 2 and adjacent regions (teleomorph of *Scedosporium apiospermum*).

GAAATGCGAT AAGTAATGTG AATTGCAGAA TTCAGTGAAT
CATCGAATCT TTGAAACGCA CATTGCGCCC GGCAGTAATC
TGCCGGGCAT GCCTGTCCGA GCGTCATTTC AACCCTCGAA
CCTCCGTTTC CTCAGGGAAG CTCAGGGTCG GTGTTGGGGC
GCTACGGCAA GTCTTCGCAA CCCTCCGTAG GCCCTGAAAT
ACAGTGGCGG TCCCGCCGCG GTTGCCTTCT GCGTAGAAGT
CTCTTTTGCA AGCTCGCATT GGGTCCCGGC GGAGGCCTGC
CGTCAAACCA CCTATAACTC CAAATGGTTT GACCTCGGAT
CAGGTAGGGT TACCCGCTGA ACTTAA (SEQ ID NO:25)

Scedosporium apiospermum (strain ATCC64215) internal transcribed spacer 2 and adjacent regions.

GAAATGCGAT AAGTAATGTG AATTGCAGAA TTCAGTGAATC
ATCGAATCTT TGAACGCACA TTGCGCCCGG CAGTAATCTG
CCGGGCATGC CTGTCCGAGC GTCATTTCAA CCCTCGAACC
TCCGTTTCCT CAGGGAAGCT CAGGGTCGGT GTTGGGGCGC
TACGGCGAGT CTTCGCGACC CTCCGTAGGC CCTGAAATAC
AGTGGCGGTC CCGCCGCGGT TGCCTTCTGC GTAGTAAGTC
TCTTTTGCAA GCTCGCATTG GGTCCCGGCG GAGGCCTGCC
GTCAAACCAC CTATAACTCC AGATGGTTTG ACCTCGGATC
AGGTAGGTAC CCGCTGAACT TAA (SEQ ID NO:26)

Scedosporium apiospermum (strain ATCC46173) internal transcribed spacer 2 and adjacent regions.

AAATGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC
ATCGAATCTT TGAACGCACA TTGCGCCCGG CAGTAATCTG
CCGGGCATGC CTGTCCGAGC GTCATTTCAA CCCTCGAACC
TCCGTTTCCT CAGGGAAGCT CAGGGTCGGT GTTGGGGCGC
TACGGCGAGT CTTCGCGACC CTCCGTAGGC CCTGAAATAC

-continued
AGTGGCGGTC CCGCCGCGGT TGCCTTCTGC GTAGTAAGTC

TCTTTTGCAA GCTCGCATTG GGTCCCGGCG GAGGCCTGCC

GTCAAACCAC CTATAACTCC AGATGGTTTG ACCTCGGATC

AGGTAGGTAC CCGCTGAACT TAA (SEQ ID NO:27)

Penicillium notatum (strain ATCC10108) internal transcribed spacer 2 and adjacent regions.

AAATGCGATA CGTAATGTGA ATTGCAAATT CAGTGAATCA

TCGAGTCTT TGAACGCACA TTGCGCCCCC TGGTATTCCG

GGGGGCATGC CTGTCCGAGC GTCATTGCTG CCCTCAAGCA

CGGCTTGTGT GTTGGGCCCC GTCCTCCGAT CCCGGGGGAC

GGGCCCGAAA GGCAGCGGCG GCACCGCGTC CGGTCCTCGA

GCGTATGGGG CTTTGTCACC CGCTCTGTAG GCCCGGCCGG

CGCTTGCCGA TCAACCCAAA TTTTTATCCA GGTTGACCTC

GGATCAGGTA GGGATACCCG CTGAACTTAA (SEQ ID NO:28)

Sporothrix schenckii (strain ATCC14284) internal transcribed spacer 2 and adjacent regions.

GAAATGCGAT ACTAATGTGA ATTGCAGAAT TCAGCGAACC

ATCGAATCTT TGAACGCACA TTGCGCCCGC CAGCATTCTG

GCGGGCATGC CTGTCCGAGC GTCATTTCCC CCCTCACGCG

CCCCGTTGCG CGCTGGTGTT GGGGCGCCCT CCGCCTGGCG

GGGGGCCCCC GAAAGCGAGT GGCGGGCCCT GTGGAAGGCT

CCGAGCGCAG TACCGAACGC ATGTTCTCCC CTCGCTCCGG

AGGCCCCCCA GGCGCCCTGC CGGTGAAAAC GCGCATGACG

CGCAGCTCTT TTTACAAGGT TGACCTCGGA TCAGGTGAGG 2

ATACCCGCTG ACTTAA (SEQ ID NO:29)

Contamination Precautions

Precautions were taken to avoid possible contamination of PCR samples by following the guidelines of Fujita and Kwok (13, 22). All buffers and distilled water used for PCR assays were autoclaved and fresh PCR reagents were aliquoted prior to use. Physical separation of laboratory areas used to prepare PCR assays and to analyze PCR products, and the use of aerosol-resistant pipette tips, reduced possible cross-contamination of samples by aerosols. Appropriate negative controls were included in each test run, including controls omitting either the primer or the DNA template during PCR assays.

Agarose gel Electrophoresis

Gel electrophoresis was conducted in TBE buffer (0.1 M Tris, 0.09 M boric acid, 1 mM EDTA, pH 8.4) at 80 V for 1 to 2 hours using gels composed of 1% (w/vol) agarose (International Technologies, New Haven, Conn.) and 1% (w/vol) NuSieve agar (FMC Bioproducts, Rockland, Me.). Gels were stained with 0.5 μg of ethidium bromide (EtBr) per ml of distilled H_2O for 10 minutes followed by three serial washes for 10 minutes each with distilled H_2O.

Microtitration Plate Enzyme Immunoassay for the Detection of PCR Products

Amplicons were detected using species-specific and genus probes labeled with digoxigenin and an all-filamentous fungal probe labeled with biotin in a streptavidin-coated microtiter plate format (13, 34). Ten μl of PCR product was added to each 1.5 ml Eppendorf tube. Single-stranded DNA was then prepared by heating the tubes at 95° C. for 5 minutes and cooling immediately on ice. Two-tenths of a ml of hybridization solution [4×SSC (saline sodium citrate buffer, 0.6 M NaCl, 0.06 M trisodium citrate, pH 7.0) containing 20 mM Hepes, 2 mM EDTA, and 0.15% (vol/vol) Tween 20] supplemented with 50 ng/ml each of the all-Aspergillus biotinylated probe and a species-specific digoxigenin-labeled probe was added to each tube containing denatured PCR product. Tubes were mixed by inversion and placed in a water bath at 37° C. to allow probes to anneal to PCR product DNA. After 1 hour, 100 μl of each sample was added to duplicate wells of a commercially prepared streptavidin-coated microtitration plate (Boehringer Mannheim, Indianapolis, Ind.). The plate was incubated at ambient temperature for 1 hour with shaking, using a microtitration plate shaker (manufactured for Dynatech by CLTI, Middletown, N.Y.). Plates were washed 6 times with 0.01 M potassium phosphate buffered saline, pH 7.2, containing 0.05% Tween 20 (PBST). Each well then received 100 μl of horseradish peroxidase-conjugated, anti-digoxigenin Fab fragment (Boehringer Mannheim) diluted 1:1000 in hybridization buffer. After incubation at ambient temperature for 30 minutes with shaking, the plate was washed 6 times with PBST. One hundred μl of a mixture of one volume of 3, 3', 5, 5'-tetramethyl benzidine peroxidase substrate (Kirkegaard and Perry Laboratories, Inc., Gaithersberg, Md.) and one volume of peroxidase solution (Kirkegaard and Perry Laboratories) was added to each well and the plate was placed at ambient temperature for 10 minutes for color development. The A_{650} nm of each well was determined with a microtitration plate reader (UV Max, Molecular Devices, Inc., Menlo Park, Calif.). The absorbance value for the reagent blank, where DNA was absent but replaced with distilled H_2O, was subtracted from each test sample.

Statistical Analysis

The Student's t test was used to determine differences between sample means. Means are expressed as the mean plus or minus the standard error from the mean. Differences were considered significant when $P<0.05$.

The following probes were used to detect and distinguish each species.

TABLE 2

PROBES	Probe Sequences 5' to 3' OLIGONUCLEOTIDE SEQUENCE	
Generic Biotin Probe	5' end-labeled biontinylated probe 5.8S region of rDNA	
B-58	GAA TCA TCG A(AG)T CTT TGA ACG	SEQ ID NO 61
Digoxigenin-probe	5' end-labeled digoxigenin probe ITS2 region of rDNA	
Aspergillus species		
A. flavus 22	GCA AAT CAA TCT TTT TCC	SEQ ID NO 30
A. flavus 23	GAA CGC AAA TCA ATC TTT	SEQ ID NO 31
A. fumigatus	CCG ACA CCC ATC TTT ATT	SEQ ID NO 32
A. niger	GAC GTT ATC CAA CCA TTT	SEQ ID NO 33
A. nidulans	GGC GTC TCC AAC CTT ATC	SEQ ID NO 35
A. terreus	GCA TTT ATT TGC AAC TTG	SEQ ID NO 34
Fusarium species		
F. moniliforme	TCT AGT GAC GGT CTC GCT	SEQ ID NO 49
F. oxysporum	CGT TAA TTC GCG TTC CTC	SEQ ID NO 50
F. solani	CTA ACA CCT CGC AAC TGG AGA	SEQ ID NO 51
Mucor species		
M. circinelloides	AAC ATT TTT GTG AAT AGG ATG	SEQ ID NO 39
M. indicus	CGT GGA TTG AGT GCC GAT	SEQ ID NO 38
M. plumbeus	GAA ACC CTT GAA ATT	SEQ ID NO 37
M. rouxii	GAA TAG GAA TAC TGA GAG	SEQ ID NO 36
M. racemosus	GAA ATC CCT GAA ATT	SEQ ID NO 40
Penicillium species		
Penicillium marneffei 1	GGG TTG GTC ACC ACC ATA	SEQ ID NO 47
Penicillium marneffei 2	TGG TCA CCA CCA TAT TTA	SEQ ID NO 48
Penicillium notatum	GAT CAA CCC AAA TTT TTA	SEQ ID NO 46
Rhizopus species		
R. circinans	CTT AGG GTA TCA TTA CTT	SEQ ID NO 42
R. microsporus	CAT ATA ATC TAG GGG TTC	SEQ ID NO 57
R. oryzae	GAG TAT AAT CCA G(CT)A ACT	SEQ ID NO 41
R. stolonifer	CTT GGT ATT ATA ACG ATT	SEQ ID NO 44
Rhizomucor pusillus	TCC TTG AGG GCT TGC ATT	SEQ ID NO 43
Other Genera		
Absidia corymbifera	GTT GCT GTC ATG GCC TTA	SEQ ID NO 55
Cunninghamella elegans 4	TAG TCG GCT TTA ATA GAT	SEQ ID NO 52
Cunninghamella elegans 5	TAT TAA GTT TAT AGC CAT	SEQ ID NO 53
Cunninghamella elegans 6	TAA GTt TAT AGC CAT TCT	SEQ ID NO 54
Pseudallescheria boydii	AAG TCT CTT TTG CAA GCT	SEQ ID NO 45
Sporothrix schoenckii	GAC GCG CAG CTC TTT TTA	SEQ ID NO 56
Genus Probes		
G-ASPERGILLUS	CCT CGA GCG TAT GGG GCT	SEQ ID NO 58
G-FUSARIUM	CCC AAC TTC TGA ATG TTG	SEQ ID NO 59
G-MUCOR	(AC)TG GGG CCT CCC AAA TAA	SEQ ID NO 60

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Species-specific probes to the ITS2 region of rDNA for *Aspergillus fumigatus* (SEQ ID NO:32), *A. flavus* (SEQ ID NO:31), *A. niger* (SEQ ID NO:33), *A. terreus* (SEQ ID NO:34), and *A. nidulans* (SEQ ID NO:35) correctly identified each of the respective species ($P<0.001$), and gave no false-positive reactions with Rhizopus, Mucor, Fusarium, Penicillium, or Candida species. The *A. flavus* probe also recognized *A. oryzae*, which belongs to the *A. flavus* group. Identification time was reduced from a mean of 5 days by conventional methods to 8 hours.

TABLE 3

	Aspergillus Probes				
Fungus	*A. fumigatus*	*A. nidulans*	*A. niger*	*A. terreus*	*A. flavus*
A. fumigatus (n = 6)	2.197 ± 0.187	0.002	0.000	0.001	0.001
A. nidulans (n = 3)	0.001	1.315 ± 0.464	0.002	0.000	0.001
A. niger (n = 5)	0.000	0.000	1.242 ± 0.471	0.001	0.003
A. terreus (n = 4)	0.001	0.000	0.001	1.603 ± 0.378	0.001
A. flavus (n = 6)	0.001	0.001	0.000	0.001	2.043 ± 0.390
A. oryzae (n = 2)	0.001	0.002	0.001	0.001	2.445 ± 0.106
A. parasitica (n = 1)	0.001	0.002	0.002	0.002	0.051
A. clavus (n = 1)	0.005	0.005	0.006	0.005	0.003
C. albicans (n = 1)	0.002	0.001	0.002	0.000	0.000
C. parasilosis (n = 1)	0.001	0.002	0.002	0.002	0.001
C. glabrata (n = 1)	0.001	0.003	0.001	0.001	0.005
C. krusei (n = 1)	0.002	0.002	0.002	0.001	0.001
C. tropicalis (n = 1)	0.002	0.002	0.001	0.000	0.001
F. moniliforme (n = 1)	0.003	0.003	0.001	0.001	0.001
F. solani (n = I)	0.006	0.002	0.001	0.000	0.001
R. oryzae (n = 1)	0.001	0.001	0.001	0.001	0.001
M. racemosus (n = 1)	0.001	0.002	0.005	0.002	0.000
P. notatum (n = 1)	0.001	0.002	0.002	0.002	0.000
Avg ± SD negative controls	0.001 ± 0.002	0.001 ± 0.001	0.000 ± 0.002	0.000 ± 0.002	0.002 ± 0.010

Species-specific probes to the ITS2 region of rDNA for *Fusarium oxysporum, F. solani*, and *F. moniliforme*, correctly identified each of the respective species (P<0.001), and gave no false-positive reactions with Blastomyces, [40] Apophysomyces, Candida, Aspergillus, Mucor, Penecillium, Rhizopus, Rhizomucor, Absidia, Cunninghamella, Pseudallescheria, Sporothrix, or Neosartorya. Empty boxes in Table 4 represent zero probe reactivity.

TABLE 4

	Fusarium Probes			
Fungus	*F. oxysporum*	*F. solani*	*F. moniliforme*	Generic Fusarium
F. oxysporum (n = 3)	1.40 ± 0.13			1.76 ± 0.27
F. solani (n = 5)		1.57 ± 0.07		1.35 ± 0.28
F. moniliforme (n = 2)			1.40 ± 0.01	1.34 ± 0.91
Negative control				
A. fumigatus				
A. flavus				
A. niger				
A. nidulans				
A. terreus				
A. parasiticus				
A. clavatus				
P. marneffei		0.01	0.01	
P. notatum	0.01	0.01	0.01	
Rhizopus oryzae		0.03	0.01	
Rhizopus microsporus		0.01	0.01	

TABLE 4-continued

	Fusarium Probes			
Fungus	*F. oxysporum*	*F. solani*	*F. moniliforme*	Generic Fusarium
Rhizopus circinans		0.01	0.01	
Rhizopus stolonifer	0.01	0.01		
Rhizomucor pusillus	0.03	0.02		
M. racemosus				
M. circinelloides				
M. rouxii				
M. plumbeus				
M. indicus				
Absidia corymbifera		0.01	0.01	
Cunninghamella elegans		0.01	0.02	
P. boydii	0.02			
Sporothrix schenckii		0.01	0.01	
C. albicans				
C. tropicalis				
C. krusei				
C. parasilosis				
C. glabrata				
Neosartorya fischeri		0.01		
Blastomyces dermatitidis				
Apophysomyces elegans				
Average of negative controls	0.001 ± 0.002	0.005 ± 0.01	0.004 ± 0.006	

Species-specific probes to various other zygomyces are presented in Table 5, showing correct identification of each species and no false positives. The exceptions are that the *M. circinelloides* probe hybridized with the *M. rouxii* DNA and the *M. plumbeus* probe hybridized with the *M. racemosus* DNA. However, the *M. rouxii* probe did not hybridize with *M. circinelloides* DNA, nor did the *M. racemosus* probe hybridize with *M. plumbeus* DNA. Therefore, by a process of elimination, each species can be correctly identified. Empty boxes in Table 5 represent zero probe reactivity.

TABLE 5

		Zygomyces Probes										
FUNGUS	D-probes RORY	RMIC	RCIR	RSTOL	RPUS	MRACE	MCIR	MRX	MPLUM	MIND	ABS	CUN
R. oryzae (n = 5)	1.50 ± 0.48				0.01							
R. microsporus (n = 5)		0.96 ± 0.61										
R. circinans (n = 3)			1.56 ± 0.19									
R. stolonifer (n = 5)				2.53 ± 0.07			0.01					
Rhizomucor pusillus (n = 2)					1.10 ± 0.68							
M. racemosus (n = 6)				0.01		2.02 ± 0.34			0.29 ± 0.52			
M. circinelloides (n = 3)							1.63 ± 0.37	0.01	0.02			
M. rouxii (n = 1)							1.77	0.76				
M. plumbeus (n = 2)									2.14 ± 0.25			
M. indicus (n = 1)		0.01							1.70 ± 0.04			
Absidia corymbifera (n = 2)					0.01			0.01		1.61 ± 0.08		
Cunninhamella elegans (n = 2)		0.01									2.26 ± 0.03	
Negative control												
A. fumigatus								0.01	0.02			
A. flavus					0.01				0.05			
A. niger						0.01						
A. nidulans								0.01	0.01			
A. terreus	0.01											
A. parasiticus					0.01				0.03			

TABLE 5-continued

	Zygomyces Probes											
FUNGUS	D-probes RORY	RMIC	RCIR	RSTOL	RPUS	MRACE	MCIR	MRX	MPLUM	MIND	ABS	CUN
A. clavatus										0.02		
P. marneffei			0.01									
P. notatum										0.03		
F. oxysporum								0.01				
F. solani												
F. moniliforme	0.01			0.01				0.01		0.01		
P. boydii	0.02											
Sporothrix schenckii												
C. albicans												
C. tropicalis												
C. krusei												
C. parasilosis												
C. glabrata												
Neosartorya fischeri			0.01									
Blastomyces dermatifidis												
Apophysomyces elegans												
Average	0.001 ± .004	0.001 ± 0.02	0.000 ± 0.002	0.000 ± 0.003	0.001 ± 0.003	0.001 ± 0.002	0.001 ± 0.002	0.001 ± 0.003	0.003 ± 0.005	0.005 ± 0.01	0.001 ± 0.001	

Species-specific probes to various other fungi are presented in Table 6, showing correct identification of each species and no false positives. Empty boxes in Table 6 represent zero probe reactivity.

TABLE 6

	Pseudallescheria and Sporothrix Probes			
Fungus	*P. boydii*	*P. marneffei*	*P. notatum*	*Sporothrix schenckii*
P. boydii (n = 4)	1.65 ± 0.48			
P. marneffei (n = 3)	0.01	1.24 ± 0.12		
P. notatum (n = 3)			1.93 ± 0.25	
Sporothrix schenckii (n = 3)	0.01			1.94 ± 0.25
Negative control				
A. fumigatus	0.01			
A. flavus				
A. niger				
A. nidulans				
A. terreus				
A. parasiticus				
A. clavatus			0.11	
F. oxysporum		0.10		
F. solani		0.14		
F. moniliforme		0.08		
R. oryzae	0.01			
R. microsporus	0.01			
R. circinans	0.01			
R. stolonifer	0.01			
Rhizomucor pusilus				
M. racemosus		0.04		
M. circinelloides	0.01	0.09		
M. rouxii	0.01			
M. plumbeus		0.05		
M. indicus				
Absidia corymbifera	0.01			
Cunninghamela bertholietiae	0.01			
C. albicans				
C. tropicalis		0.02		
C. krusei				
C. parasilosis				
C. glabrata				
Neosatorya pseudofischeri		0.03		
Blastomyces dermatitidis	0.01			

TABLE 6-continued

	Pseudallescheria and Sporothrix Probes			
Fungus	*P. boydii*	*P. marneffei*	*P. notatum*	*Sporothrix schenckii*
Apophysomyces elegans	0.01			
Average Negative Controls	0.004 ± 0.002	0.013 ± 0.03	0.002 ± 0.019	0.001 ± 0.002

All of the references mentioned in this Specification are hereby incorporated by reference in their entirety.

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ctaaaataca gcctctttgt aattttcatt aaattacgaa ctacctagcc atcgtgcttt 240

tttggtccaa ccaaaaaaca tataatctag gggttctgct agccagcaga tattttaatg 300

atctttaact atgatctgaa gtcaagtggg actacccgct gaacttaa 348

<210> SEQ ID NO 16
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Rhizopus microsporus

<400> SEQUENCE: 16

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tgcactctat ggatcttcta tagagtacgc ttgcttcagt atcataacca acccacacat 120

aaaatttatt ttatgtggtg atggacaagc tcggttaaat ttaattatta taccgattgt 180

ctaaaataca gcctctttgt aattttcatt aaattacgaa ctacctagcc atcgtgcttt 240

tttggtccaa ccaaaaaaca tataatctag gggttctgct agccagcaaa tattttaatg 300

atctttaacc tatgatctga agtcaagtgg gactacccgc tgaacttaa 349

<210> SEQ ID NO 17
<211> LENGTH: 361
<212> TYPE: DNA
<213> ORGANISM: Rhizopus circinans

<400> SEQUENCE: 17

aaattgcgat aactagtgtg aattgcattt tcagtgaatc atcgagtctt tgaacgcatc 60

ttgcgctctt gggattcttc cctagagcac acttgcttca gtatcataac aaaaccctca 120

cctaatattt tttttttta aaaaaaaaat attagagtgg tattggggtc tctttggtaa 180

ttctttgtaa ttataaaagt acccttaaat gtcataaaca ggttagcttt agcttgcctt 240

taaagatctt cttagggtat cattactttt cgtaaatctt taataggcct gtcacataat 300

tctaccctta aatttcttaa accttgatct gaagtcaagt gggagtaccc gctgaactta 360

a 361

<210> SEQ ID NO 18
<211> LENGTH: 360
<212> TYPE: DNA
<213> ORGANISM: Rhizopus circinans

<400> SEQUENCE: 18

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ttgcgctctt gggattcttc cctagagcac acttgcttca gtatcataac aaaaccctca 120

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cctaatattt ttttttaaaa aaaaaaaata ttagagtggt attggggtct ctttggtaat 180

tctttgtaat tataaaagta cccttaaatg tcataaacag gttagcttta gcttgccttt 240

aaagatcttc ttagggtatc attacttttc gtaaatcttt aataggcctg tcacataatt 300

ctacccttaa atttcttaaa ccttgatctg aagtcaagtg ggagtacccg ctgaacttaa 360

<210> SEQ ID NO 19
<211> LENGTH: 486
<212> TYPE: DNA
<213> ORGANISM: Rhizopus stolonifer

<400> SEQUENCE: 19

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ttgcactcta tggttttccg taaagtacgc ttgcttcagt atcataaaga ccccatcctg 120

attattattt ttttattaaa ataattaatt ttggagataa taaaaatgag gctctttctt 180

ttctttttt ttttttaaa aaaaaggggg ggaaagggtc ttttaaaatg ggcaaattct 240

gggtttttta ctaaacctga actcccccca aaaattcaaa aaaaaaaaaa tgggttttac 300

caaatttttt ttttttttct ccttttgtg tagttaatac tctattaaat ttatttactt 360

ggtattataa cgattatgca agaagggaga gaacaaagaa taatgaaaga gagttttaa 420

ataaattctt ttttcatttt ttcaatcaat gatctgaagt caagtgggat tacccgctga 480

acttaa 486

<210> SEQ ID NO 20
<211> LENGTH: 349
<212> TYPE: DNA
<213> ORGANISM: Rhizomucor pusillus

<400> SEQUENCE: 20

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ttgcaccctt tggttcatcc attgggtacg tctagttcag tatctttatt aacccctaaa 120

ggtttatttt ttgataaatc tttggatttg cggtgctgat ggattttcat ccgttcaagc 180

tacccgaaca atttgtatgt tgttgaccct tgatatttcc ttgagggctt gcattggtat 240

ctaatttttt accagtgtgc ttcgagatga tcaagtataa aggtcaatca accacaaata 300

aatttcaact atggatctga acttagatgg gattacccgc tgaacttaa 349

<210> SEQ ID NO 21
<211> LENGTH: 425
<212> TYPE: DNA
<213> ORGANISM: Absidia corymbifera

<400> SEQUENCE: 21

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ttgcgcctag tagtcaatct actaggcaca gttgtttcag tatctgcaac taccaatcag 120

ttcaacttgg ttctttgaac ctaagcgagc tggaaatggg cttgtgttga tggcattcag 180

ttgctgtcat ggccttaaat acatttagtc ctaggcaatt ggctttagtc atttgccgga 240

tgtagactct agagtgcctg aggagcaacg acttggttag tgagttcata attccaagtc 300

aatcagtctc ttcttgaact aggtcttaat ctttatggac tagtgagagg atctaacttg 360

ggtcttctct taaaacaaac tcacatctag atctgaaatc aactgagatc acccgctgaa 420

cttaa 425

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<210> SEQ ID NO 22
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Absidia corymbifera

<400> SEQUENCE: 22

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ttgcgcctag tagtcaatct actaggcaca gttgtttcag tatctgcatc caccaatcaa 120

cttaaccttt tgtgttgagt tggaactggg cttctagttg atggcattta gttgctgtca 180

tggccttaaa tcaatgtcct aggtgttaga acatctaaca ccggatggaa actttagagc 240

gctttaagag cagcttggtt agtgagttca ataattccaa gcattaagtc ttttaatgaa 300

ctagcttttc tatctatggg acactacttg gagaaatcca agtaaccttt aaactcccat 360

ttagatctga aatcaactga gaccacccgc tgaacttaa 399

<210> SEQ ID NO 23
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<212> TYPE: DNA
<213> ORGANISM: Cunninghamella elegans

<400> SEQUENCE: 23

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gcaccttatg gtattccata aggtacgtct gtttcagtac cactaataaa tctctctcta 120

tccttgatga tagaaaaaaa aaaaataatt tttactgggc ccggggaatc ctttttttt 180

tttaataaaa aggaccaatt ttggcccaaa aaaaagggtt gaacttttt taccagatct 240

tgcatctagt aaaaacctag tcggctttaa tagattttta ttttctatta agtttatagc 300

cattcttata ttttttaaaa tcttggcctg aaatcagatg ggatacccgc tgaacttaa 359

<210> SEQ ID NO 24
<211> LENGTH: 346
<212> TYPE: DNA
<213> ORGANISM: Pseudallescheria boydii

<400> SEQUENCE: 24

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attgcgcccg gcagtaatct gccgggcatg cctgtccgag cgtcatttca accctcgaac 120

ctccgtttcc ttagggaagc ctagggtcgg tgttggggcg ctacggcaag tcctcgcaac 180

ccccgtaggc cctgaaatac agtggcggtc ccgccgcggt tgccttctgc gtagtaagtc 240

tcttttgcaa gctcgcattg ggtcccggcg gaggcctgcc gtcaaaccac ctaacaactc 300

cagatggttt gacctcggat caggtagggt tacccgctga acttaa 346

<210> SEQ ID NO 25
<211> LENGTH: 346
<212> TYPE: DNA
<213> ORGANISM: Pseudallescheria boydii

<400> SEQUENCE: 25

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cattgcgccc ggcagtaatc tgccgggcat gcctgtccga gcgtcatttc aaccctcgaa 120

cctccgtttc ctcagggaag ctcagggtcg gtgttggggc gctacggcaa gtcttcgcaa 180

ccctccgtag gccctgaaat acagtggcgg tcccgccgcg gttgccttct gcgtagaagt 240

ctcttttgca agctcgcatt gggtcccggc ggaggcctgc cgtcaaacca cctataactc 300

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caaatggttt gacctcggat caggtagggt tacccgctga acttaa 346

<210> SEQ ID NO 26
<211> LENGTH: 344
<212> TYPE: DNA
<213> ORGANISM: Scedosporium apiospermum

<400> SEQUENCE: 26

gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgaatct ttgaacgcac 60

attgcgcccg gcagtaatct gccgggcatg cctgtccgag cgtcatttca accctcgaac 120

ctccgtttcc tcagggaagc tcagggtcgg tgttggggcg ctacggcgag tcttcgcgac 180

cctccgtagg ccctgaaata cagtggcggt cccgccgcgg ttgccttctg cgtagtaagt 240

ctcttttgca agctcgcatt gggtcccggc ggaggcctgc cgtcaaacca cctataactc 300

cagatggttt gacctcggat caggtaggta cccgctgaac ttaa 344

<210> SEQ ID NO 27
<211> LENGTH: 343
<212> TYPE: DNA
<213> ORGANISM: Scedosporium apiospermum

<400> SEQUENCE: 27

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ttgcgcccgg cagtaatctg ccgggcatgc ctgtccgagc gtcatttcaa ccctcgaacc 120

tccgtttcct cagggaagct cagggtcggt gttggggcgc tacggcgagt cttcgcgacc 180

ctccgtaggc cctgaaatac agtggcggtc ccgccgcggt tgccttctgc gtagtaagtc 240

tcttttgcaa gctcgcattg ggtcccggcg gaggcctgcc gtcaaaccac ctataactcc 300

agatggtttg acctcggatc aggtaggtac ccgctgaact taa 343

<210> SEQ ID NO 28
<211> LENGTH: 309
<212> TYPE: DNA
<213> ORGANISM: Penicillium notatum

<400> SEQUENCE: 28

aaatgcgata cgtaatgtga attgcaaatt cagtgaatca tcgagtcttt gaacgcacat 60

tgcgcccccc ggtattccgg ggggcatgcc tgtccgagcg tcattgctgc cctcaagcac 120

ggcttgtgtg ttgggccccg tcctccgatc ccgggggacg ggcccgaaag gcagcggcgg 180

caccgcgtcc ggtcctcgag cgtatggggc tttgtcaccc gctctgtagg cccggccggc 240

gcttgccgat caacccaaat ttttatccag gttgacctcg gatcaggtag ggatacccgc 300

tgaacttaa 309

<210> SEQ ID NO 29
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Sporothrix schenckii

<400> SEQUENCE: 29

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ttgcgcccgc cagcattctg gcgggcatgc ctgtccgagc gtcatttccc ccctcacgcg 120

ccccgttgcg cgctggtgtt ggggcgccct ccgcctggcg gggggccccc gaaagcgagt 180

ggcgggccct gtggaaggct ccgagcgcag taccgaacgc atgttctccc ctcgctccgg 240

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aggcccccca ggcgccctgc cggtgaaaac gcgcatgacg cgcagctctt tttacaaggt 300

tgacctcgga tcaggtgagg atacccgctg acttaa 336

<210> SEQ ID NO 30
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus flavus

<400> SEQUENCE: 30

gcaaatcaat cttttttcc 18

<210> SEQ ID NO 31
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus fumigatus

<400> SEQUENCE: 31

gaacgcaaat caatcttt 18

<210> SEQ ID NO 32
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus fumigatus

<400> SEQUENCE: 32

ccgacaccca tctttatt 18

<210> SEQ ID NO 33
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus niger

<400> SEQUENCE: 33

gacgttatcc aaccattt 18

<210> SEQ ID NO 34
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus terreus

<400> SEQUENCE: 34

gcatttattt gcaacttg 18

<210> SEQ ID NO 35
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus nidulans

<400> SEQUENCE: 35

ggcgtctcca accttatc 18

<210> SEQ ID NO 36
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mucor rouxii

<400> SEQUENCE: 36

gaataggaat actgagag 18

<210> SEQ ID NO 37
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mucor indicus

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<400> SEQUENCE: 37

gaaacccttg aaatt 15

<210> SEQ ID NO 38
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mucor indicus

<400> SEQUENCE: 38

cgtggattga gtgccgat 18

<210> SEQ ID NO 39
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mucor circinelloides f.

<400> SEQUENCE: 39

aacatttttg tgaataggat g 21

<210> SEQ ID NO 40
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Mucor racemosus

<400> SEQUENCE: 40

gaaatccctg aaatt 15

<210> SEQ ID NO 41
<211> LENGTH: 18
<212> TYPE: DNA
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<400> SEQUENCE: 41

gagtataatc cagyaact 18

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Rhizopus circinans

<400> SEQUENCE: 42

cttagggtat cattactt 18

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Rhizomucor pusillus

<400> SEQUENCE: 43

tccttgaggg cttgcatt 18

<210> SEQ ID NO 44
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Rhizopus stolonifer

<400> SEQUENCE: 44

cttggtatta taacgatt 18

<210> SEQ ID NO 45
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: Pseudallescheria boydii

<400> SEQUENCE: 45

aagtctcttt tgcaagct 18

<210> SEQ ID NO 46
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Penicillium notatum

<400> SEQUENCE: 46

gatcaaccca aattttta 18

<210> SEQ ID NO 47
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Penicillium marneffei

<400> SEQUENCE: 47

gggttggtca ccaccata 18

<210> SEQ ID NO 48
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Penicillium marneffei

<400> SEQUENCE: 48

tggtcaccac catattta 18

<210> SEQ ID NO 49
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Fusarium moniliforme

<400> SEQUENCE: 49

tctagtgacg gtctcgct 18

<210> SEQ ID NO 50
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 50

cgttaattcg cgttcctc 18

<210> SEQ ID NO 51
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Fusarium solani

<400> SEQUENCE: 51

ctaacacctc gcaactggag a 21

<210> SEQ ID NO 52
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Cunninghamella elegans

<400> SEQUENCE: 52

tagtcggctt taatagat 18

<210> SEQ ID NO 53
<211> LENGTH: 18

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<212> TYPE: DNA
<213> ORGANISM: Cunninghamella elegans

<400> SEQUENCE: 53

tattaagttt atagccat 18

<210> SEQ ID NO 54
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Cunninghamella elegans

<400> SEQUENCE: 54

taagtttata gccattct 18

<210> SEQ ID NO 55
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Absidia corymbifera

<400> SEQUENCE: 55

gttgctgtca tggcctta 18

<210> SEQ ID NO 56
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Sporothrix schenckii

<400> SEQUENCE: 56

gacgcgcagc tcttttta 18

<210> SEQ ID NO 57
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Rhizopus microsporus

<400> SEQUENCE: 57

catataatct aggggttc 18

<210> SEQ ID NO 58
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Aspergillus sp.

<400> SEQUENCE: 58

cctcgagcgt atggggct 18

<210> SEQ ID NO 59
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Fusarium sp.

<400> SEQUENCE: 59

cccaacttct gaatgttg 18

<210> SEQ ID NO 60
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Mucor sp.

<400> SEQUENCE: 60

mtggggcctc ccaaataa 18

<210> SEQ ID NO 61

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<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: B-58 biotin probe

<400> SEQUENCE: 61

gaatcatcga rtctttgaac g 21

We claim:

1. An isolated nucleic acid probe that consists essentially of 10 to 50 consecutive nucleotides for species-specific identification of Aspergillus, wherein the probe selectively hybridizes under stringent conditions to the internal transcribed spacer 2 nucleic acid sequence of one of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* (SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5), but does not selectively hybridize under stringent conditions to the internal transcribed spacer 2 region of any other Aspergillus species, nor does it hybridize to the internal transcribed spacer 2 nucleic acid sequence of *Fusarium solani* (SEQ ID NO:6), *Fusarium moniliforme* (SEQ ID NO:7), *Mucor rouxii* (SEQ ID NO:8), *Mucor racemosus* (SEQ ID NO:9), *Mucor plumbeus* (SEQ ID NO:10), *Mucor indicus* (SEQ ID NO:11), *Mucor circinilloides f. circinelloides* (SEQ ID NO:12), *Rhizopus oryzae* (SEQ ID NO:13 and NO:14), *Rhizopus microsporus* (SEQ ID NO:15 and 16), *Rhizopus circinans* (SEQ ID NO:17 and 18), *Rhizopus stolonifer* (SEQ ID NO:19), *Rhizomucor pusillus* (SEQ ID NO:20), *Absidia corymbifera* (SEQ ID NO:21 and 22), *Cunninghamella elegans* (SEQ ID NO:23), *Pseudallescheria boydii* (teleomorph of *Scedosporium apiospermum*) (SEQ ID NO:24, 25, 26, and 27), *Penicillium notatum* (SEQ ID NO:28), or *Sporothrix schenkii* (SEQ ID NO:29).

2. The isolated nucleic acid probe of claim **1** wherein the probe selectively hybridizes with an *Aspergillus flavus* nucleic acid of SEQ ID NO:1, or a complementary sequence thereof.

3. The isolated nucleic acid probe of claim **1** wherein the probe selectively hybridizes with an *Aspergillus fumigatus* nucleic acid of SEQ ID NO:2, or a complementary sequence thereof.

4. The isolated nucleic acid probe of claim **1** wherein the probe selectively hybridizes with an *Aspergillus niger* nucleic acid of SEQ ID NO:3, or a complementary sequence thereof.

5. The isolated nucleic acid probe of claim **1** wherein the probe selectively hybridizes with an *Aspergillus terreus* nucleic acid of SEQ ID NO:4, or a complementary sequence thereof.

6. The isolated nucleic acid probe of claim **1** wherein the probe selectively hybridizes with an *Aspergillus nidulans* nucleic acid of SEQ ID NO:5, or a complementary sequence thereof.

7. A method of detecting a species of *Aspergillus flavus* (SEQ ID NO:1), *Aspergillus fumigatus* (SEQ ID NO:2), *Aspergillus niger* SEQ ID NO:3), *Aspergillus terreus* (SEQ ID NO:4), or *Aspergillus nidulans* (SEQ ID NO:5) in a sample comprising

contacting the sample with a nucleic acid probe consisting essentially of 10 to 50 consecutive nucleotides that selectively hybridizes with a nucleic acid having a sequence as set forth as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5, or a complementary sequence thereof;

wherein hybridization of the nucleic acid probe with the sample indicates the detection of the Aspergillus species in the sample.

8. The method of claim **7**, wherein the probe selectively hybridizes with an *Aspergillus flavus* nucleic acid of SEQ ID NO:1, or a complementary sequence thereof.

9. The method of claim **7**, wherein the probe selectively hybridizes with an *Aspergillus fumigatus* nucleic acid of SEQ ID NO:2, or a complementary sequence thereof.

10. The method of claim **7**, wherein the probe selectively hybridizes with an *Aspergillus niger* nucleic acid of SEQ ID NO:3, or a complementary sequence thereof.

11. The method of claim **7**, wherein the probe selectively hybridizes with an *Aspergillus terreus* nucleic acid of SEQ ID NO:4, or a complementary sequence thereof.

12. The method of claim **7**, wherein the probe selectively hybridizes with an *Aspergillus nidulans* nucleic acid of SEQ ID NO:5, or a complementary sequence thereof.

13. An isolated nucleic acid probe for identifying a filamentous fungus wherein the probe consists essentially of a nucleic acid having a sequence as set forth as SEQ ID NO:61, or a complementary sequence thereof, respectively.

14. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of a nucleotide sequence having a sequence as set forth as SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, or SEQ ID NO:35.

15. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:30.

16. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:31.

17. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:32.

18. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:33.

19. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:34.

20. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:35.

21. The method of claim **7**, wherein the probe consists essentially of a nucleotide sequence as set forth as SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

22. The method of claim **7**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:30.

23. The method of claim **7**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:31.

24. The method of claim **7**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:32.

25. The method of claim **7**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:33.

26. The method of claim **7**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:34.

27. The isolated nucleic acid probe of claim **1**, wherein the probe consists essentially of the nucleotide sequence set forth as SEQ ID NO:35.

28. An isolated nucleic acid comprising a sequence as set forth as SEQ ID NO:1 or SEQ ID NO:2.

29. An isolated nucleic acid consisting essentially of a sequence as set forth as SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5.

* * * * *